

The effectiveness of preplant seed bio-invigoration techniques using *Bacillus* sp. CKD061 to improving seed viability and vigor of several local upland rice cultivars of Southeast Sulawesi

G A K Sutariati^{1*}, L O S Bande¹, A Khaeruni¹, Muhidin¹, L Mudi¹ and R M Savitri²

¹Department of Agrotechnology, Faculty of Agriculture, Universitas Halu Oleo, Kendari Southeast Sulawesi 93212 Indonesia

²Graduated Student at Department of Agrotechnology, Faculty of Agriculture, Universitas Halu Oleo, Kendari Southeast Sulawesi 93212 Indonesia

Email : sutariati69@yahoo.co.id

Abstract. Research was aimed to evaluate the bio-invigoration techniques using *Bacillus* sp. CKD061 in improving seed viability and vigor of local upland rice. The research is arranged in factorial with completely randomized design (CRD). The different upland rice cultivars as first factor that consists of 11 cultivars, namely: *Pae Tinangge*, *Pae Rowu*, *Pae Uwa*, *Pae Tanta*, *Pae Waburi-Buri*, *Pae Mornene*, *Pae Indalibana*, *Pae Lawarangka*, *Pae Huko*, *Pae Wagamba* and *Pae Momea*. The second factor is the seed bio-invigoration technique, consists of 5 treatments, namely: without seed bio-invigoration (B₀), NaCl + *Bacillus* sp. CKD061 (B₁), KNO₃ + *Bacillus* sp. CKD061 (B₂), Ground burned-rice husk + *Bacillus* sp. CKD061 (B₃), and Ground brick + *Bacillus* sp. CKD061 (B₄). The results showed that seed bio-invigoration using *Bacillus* sp. CKD061 gave effect on the seed viability and vigor. Interaction of the seed bio-invigoration and upland rice cultivars were able to improve seed viability and vigor. Seed bio-invigoration treatment using ground brick + *Bacillus* sp. CKD061 was the best treatment, which could improve the viability and vigor of *Pae Waburi-Buri*, *Pae Mornene* and *Pae Indalibana*. The treatment increased vigor index by 133% in *Pae Waburi-Buri* and 127% in *Pae Mornene*, and *Pae Indalibana* compared with control.

1. Introduction

Rice (*Oryza sativa* L.) is a very important food crop because rice is still the main staple food in Indonesian. The demand of rice is increase every year. Various efforts have been done to increase rice production such as through the increasing productivity, breeding new variety [1] and development rice under the shade [2]. Another program is by the decreasing the level of rice consumption and promoting a local source of staple foods such sago [3], cassava or corn [4], but the results are not yet optimal. One effort to rice production is through the development of upland rice in dry land [4].

Constraints encountered include limited input of cultivation technology, especially in terms of the use of quality seeds and control techniques of plant-disturbing organisms. In addition, the decline in rice production is also attributed to the decrease in productive wetland due to the transfer of functions to the interests of industry, housing and other non-agricultural land use. The development of upland rice in dry land can be a solution to increase production. However, upland rice received less attention because of its low productivity. Development of upland rice (especially local upland rice) in Southeast



Sulawesi is still limited, in addition to marginal land issues, as well as the implementation of cultivation techniques of upland rice, especially in the use of quality seeds. The use of high quality seeds is an important prerequisite for generating economically profitable crop production. Therefore, preparation and treatment of seeds to improve the quality is very important to do, especially with the physiological dormancy (after ripening) problem in post-harvest upland rice seed in the field.

The alternative to overcome these problems is through seed invigoration technology integrated with biological agents of rhizobacterial groups, microorganisms capable of acting as biofertilizers and biopesticides [5]. Invigoration is a way to improve the physiological quality of seeds, especially seed vigor, through physical or chemical treatment. High-vigorous seeds are able to demonstrate good performance in germination processes under diverse environmental conditions [6]. Seed invigoration is physiological and biochemical improvements associated with synchronous germination, velocity, and increased seed germination using low-potency matrix solids or low osmotic potential solutions. This treatment is known as seed matricconditioning or osmoconditioning treatments can be integrated with rhizobacterial applications, called bio-matricconditioning [7-8] or bio-osmoconditioning. This treatment aims to improve the viability and vigor of seeds, growth and yield of plants [5,9], also proved able to protect the seed from seedborne and soilborne fungi at an important phase at the beginning of its growth [10]. Treatment can be recommended as a growth promoting of local rice crops of Southeast Sulawesi.

2. Materials and Methods

The research was conducted in Agrotechnology Laboratory, Agriculture Faculty of Halu Oleo University, from September 2015 to March 2016. The research was arranged based on factorial in completely randomized design (CRD). The first factor is the local upland rice cultivar consisting of 11 local upland rice cultivar of Southeast Sulawesi: V1=*Pae Tinangge*, V2=*Pae Rowu*, V3=*Pae Uwa*, V4=*Pae Tanta*, V5=*Pae Waburi-Buri*, V6=*Pae Mornene*, V7=*Pae Indalibana*, V8=*Pae Lawarangka*, V9=*Pae Huko*, V10 =*Pae Wagamba* dan V11=*Pae Momea*. The second factor is bio-invigoration treatment with *Bacillus* sp. CKD061 Consists of 5 treatments namely: B₀ = without bio-invigoration treatment (as control), B₁ = NaCl + *Bacillus* sp. CKD061, B₂ = KNO₃ + *Bacillus* sp. CKD061, B₃ = ground burned-rice husk + *Bacillus* sp. CKD061 dan B₄ = ground brick + *Bacillus* sp. CKD061. Each treatment was replicated 3 times, therefore, overall there were 165 experimental units.

The effects of seed bio-invigoration on the seed viability and vigor were evaluated by measuring their germination percentage, vigor index, relative growth rate, and growth uniformity.

1. Germination percentage (GP), depicting seed potential viability [11], was measured based on the percentage of normal seedlings (NS) during the first (i.e. 5 days after planting (dap)) and the second (i.e. 7 dap) observation by using the following formula:

$$GP = \frac{\sum \text{NS at observation 1} + \sum \text{NS observation 2}}{\sum \text{seeds planted}} \times 100\%$$

2. Relative growth rate (RG-r), depicting seed vigor, is the ratio of KCT to maximum RG-r. The maximum RG itself was obtained from the assumption that at the first observation, normal seedlings had reached 100%.
3. Seed uniformity, depicting seed vigor, was measured based on the percentage of normal seedlings (NS) on the day between the first count (5 dap) and second (7 dap) i.e. at 6 dap.
4. Vigor index (VI), depicting the growth rate vigor [10], was measured based on percentage of normal seedlings at the first observation (i.e. 5 dap):

$$VI = \frac{\sum \text{NS at observation 1}}{\sum \text{seeds planted}} \times 100\%$$

3. Results and discussion

3.1. Results

3.1.1. The effects of seed bio-invigoration treatment on germination percentage on local upland.

There is an interaction effect between seed bio-invigoration treatment and cultivars on the germination percentage (Table 1). Different cultivars show different responses to the seed bio-invigoration treatment. In *Pae Uwa* cultivar the best interaction is in the treatment of $\text{KNO}_3 + \text{Bacillus}$ sp. CKD061 and ground burned rice husk + *Bacillus* sp. CKD061 with the value of each germination percentage is 94.67% and 93.33%. In *Pae Waburi-buri* cultivar the best interaction is in the treatment of $\text{NaCl} + \text{Bacillus}$ sp. CKD061, $\text{KNO}_3 + \text{Bacillus}$ sp. CKD061, ground burned rice husk + *Bacillus* sp. CKD061, ground brick + *Bacillus* sp. CKD061 with the value of each germination percentage is 93.33%, 93.33%, 94.67% and 93.33%.

Table 1. The effects of seed bio-invigoration treatment on germination percentage of several local upland rice cultivars of Southeast Sulawesi

Local upland rice cultivar	Seed Bio-invigoration Treatment									
	B0		B1		B2		B3		B4	
V1	65.33	abQ	64.67	bQ	81.33	abP	72.00	bPQ	78.67	abPQ
V2	50.67	bQ	83.33	abP	54.67	cQ	84.00	abP	62.67	bQ
V3	69.33	aQ	81.33	abPQ	94.67	aP	93.33	aP	81.33	abPQ
V4	13.33	cR	84.00	abP	61.33	bcQ	69.33	bQ	67.33	bQ
V5	65.33	abQ	93.33	aP	93.33	aP	94.67	aP	93.33	aP
V6	61.33	abQ	60.00	bQ	80.00	abPQ	73.33	bQ	90.67	aP
V7	61.33	abQ	60.00	bQ	80.00	abPQ	73.33	bQ	90.67	aP
V8	65.33	abQ	78.67	abPQ	77.33	bPQ	73.33	bPQ	84.00	aP
V9	57.33	abQ	78.67	abP	69.33	bcPQ	78.67	abP	64.00	bPQ
V10	73.33	aP	80.00	abP	86.67	abP	89.33	aP	88.00	aP
V11	61.33	abQ	73.33	bPQ	77.33	bP	73.33	bPQ	84.00	aP

Means in the same column (a-c) or in the same line (P-Q) suffixed with different letters are different at 5% levels of significance according to DMRT. V1=*Pae Tinangge*, V2=*Pae Rowu*, V3=*Pae Uwa*, V4=*Pae Tanta*, V5=*Pae Waburi-Buri*, V6=*Pae Mornene*, V7=*Pae Indalibana*, V8=*Pae Lawarangka*, V9=*Pae Huko*, V10 =*Pae Wagamba*, V11=*Pae Momea*, B0 = control, B1 = $\text{NaCl} + \text{Bacillus}$ sp. CKD061, B2 = $\text{KNO}_3 + \text{Bacillus}$ sp. CKD061, B3 = ground burned rice husk + *Bacillus* sp. CKD061, B4 = ground brick + *Bacillus* sp. CKD061

In *Pae Wagamba* cultivar the best interaction is in the treatment of ground burned rice husk + *Bacillus* sp. CKD061, ground brick + *Bacillus* sp. CKD061 with the value of each germination percentage is 89.33% dan 88.00%. Meanwhile, *Pae Mornene*, *Pae Indalibana*, *Pae Lawarangka*, and *Pae Momea*, provides the best response to the treatment of ground brick + *Bacillus* sp. CKD061 with the value of each germination percentage is 90.67%, 90.67, 84.00% and 84.00%.

3.1.2. The effects of seed bio-invigoration treatment on germination growth rate on local upland rice.

Pae Waburi-Buri, *Pae Mornene*, and *Pae Indalibana* cultivars provide a better response to the treatment of ground brick + *Bacillus* sp. CKD061 with the value of each germination percentage is 93.33%, 90.67% dan 90.67%. Among all of seed bio-invigoration treatments tested, *Pae Waburi-Buri* cultivar was able to respond all of seed bio-invigoration treatments provided. Among all of seed bio-invigoration treatments tested, only $\text{NaCl} + \text{Bacillus}$ sp. CKD061 was able to solve the dormancy of *Pae Tanta* cultivars by increasing the relative growth rate by 565% (Table 2).

Table 2. The effects of seed bio-invigoration treatment on relative growth rate of several local upland rice cultivars of Southeast Sulawesi

Local upland rice cultivar	Seed Bio-invigoration Treatment									
	B0		B1		B2		B3		B4	
V1	59.90	abQ	64.35	bPQ	78.22	abP	67.56	bPQ	66.67	bcPQ
V2	43.11	bQ	81.27	aP	47.08	cQ	74.03	bP	54.70	cQ
V3	67.17	abQ	79.68	abPQ	91.68	aP	88.92	abP	77.46	bPQ
V4	12.29	cR	81.75	aP	59.52	cQ	68.35	bPQ	65.56	bcQ
V5	58.51	abQ	86.54	aP	92.95	aP	94.29	aP	93.33	aP
V6	57.40	abQ	60.41	bQ	80.00	abPQ	70.06	bQ	90.67	aP
V7	57.40	abQ	60.41	bQ	80.00	abPQ	70.06	bQ	90.67	aP
V8	62.05	abQ	76.38	abP	76.79	bP	72.38	bPQ	80.83	abP
V9	49.56	bQ	76.48	abP	62.86	bcPQ	76.29	bP	63.17	bcPQ
V10	70.67	aQ	77.59	abPQ	82.51	abPQ	88.73	abP	85.68	abP
V11	60.19	abQ	75.24	abP	74.79	bP	72.38	bPQ	80.83	abP

Notes: Means in the same column (a-c) or in the same line (P-Q) suffixed with different letters are different at 5% levels of significance according to DMRT.

3.1.3. The effects of seed bio-invigoration treatment on vigor index of several local upland rice cultivars of Southeast Sulawesi.

Cultivars of *Pae Waburi-Buri*, *Pae Mornene*, and *Pae Indalibana* provide a better response to the treatment of ground brick + *Bacillus* sp. CKD061 with value vigor index respectively 93.33%, 90.67% and 90.67%. Consistently, seed bio-invigoration treatment using NaCl + *Bacillus* sp. CKD061 was able to solve the dormancy of *Pae Tanta* cultivars by increasing the vigor index by 817% (Table 3).

Table 3. Effect of interaction bio-invigoration technique interaction on seeds vigor several cultivars of local rice seedlings of Southeast Sulawesi

Local upland rice cultivar	Bio-invigoration									
	B0		B1		B2		B3		B4	
V1	41.33	aQ	41.33	bQ	69.33	bP	45.33	cQ	40.00	cQ
V2	18.67	bQ	60.00	aP	32.00	cQ	34.67	cQ	32.00	cQ
V3	57.33	aQ	66.67	aPQ	78.67	abP	70.67	bPQ	60.00	bQ
V4	8.00	bR	73.33	aP	53.33	bcQ	65.33	bPQ	60.00	bPQ
V5	38.67	aR	64.00	aQ	92.00	aP	93.33	aP	93.33	aP
V6	45.33	aQ	58.67	abQ	80.00	abP	61.33	bcQ	90.67	aP
V7	45.33	aQ	58.67	abQ	80.00	abP	61.33	bcQ	90.67	aP
V8	53.33	aQ	70.00	aPQ	76.00	abP	66.67	bPQ	60.00	bPQ
V9	17.33	bR	69.33	aP	40.00	cQ	65.33	bP	60.00	bP
V10	57.33	aQ	62.67	aQ	69.33	bPQ	86.67	abP	76.00	abPQ
V11	57.33	aQ	70.67	aQ	75.33	abP	66.67	bPQ	70.67	bPQ

Notes: Means in the same column (a-c) or in the same line (P-R) suffixed with different letters are different at 5% levels of significance according to DMRT.

3.2. Discussion

Seed bio-invigoration treatment using *Bacillus* sp. CKD061 integrated with ground burned rice husk or ground brick or KNO₃ gives better results in enhancing the viability and vigor of local upland rice seed compared with NaCl and control. *Bacillus* spp. is a group of PGPR (Plant Growth Promoting Rhizobacteria) which has been shown to be effective in increasing plant growth and yield [12]. The role of PGPR in increasing plant growth and production was presumably caused by the ability of rhizobacteria to produce IAA [9], gibberellins [13] and to dissolve phosphate [5,14]. In general, the utilization of *Bacillus* sp. CKD061 integrated with matricconditioning of ground burned rice husk or ground brick resulted in more effective in increasing the viability and vigor of local upland rice seed

4. Conclusions

It is concluded that seeds bio-invigoration treatment using *Bacillus* sp. CKD061 which is integrated with a medium of ground burned rice husk or ground brick or KNO₃ solution, is better able to increase the viability and vigor of local upland rice seed compared to other treatments and controls. Cultivars *Pae Waburi-Buri*, *Pae Mornene*, and *Pae Indalibana* responded better to the treatment of ground brick + *Bacillus* sp. CKD061 through increased vigor index respectively 131%, 100% and 100%. Integration of bio-invigoration seed treatment using NaCl + *Bacillus* sp. CKD061 was able to overcome the dormancy of local upland rice seed cv. *Pae Tanta*.

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